THE
AMERICAN NATURALIST

VOL. L. *October, 1916* No. 598

A NOTE ON THE INHERITANCE OF EYE PATTERN IN BEANS AND ITS RELATION TO TYPE OF VINE[1]

FRANK M. SURFACE

IN a recent paper (Pearl and Surface, 1915) from this laboratory two varieties of yellow-eyed beans were described and figured under the somewhat provincial names of Improved Yellow Eye and Old-Fashioned Yellow Eye. The type of eye pattern characteristic of each of these varieties is shown below in Figs. 1 and 2. On the Improved Yellow Eye the colored area covers about one fourth the area of the bean. The outer border of the eye pattern is clear-cut and regular, with very little or no spotting on the remainder of the bean.

The Old-Fashioned Yellow Eye pattern (Fig. 2) is much smaller in area and is quite irregular in outline but nevertheless very definite. It consists of at least three color centers: (1) A posterior[2] spot covering the caruncle and extending at least part way around the hilum. Laterally this area is extended into two rather broad wings which reach as far forward as the micropyle. (2) An anterior spot surrounding the micropyle, and (3) an anterior stripe which may or may not connect with the micropyle spot.

In connection with other work a number of crosses have

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 99.

[2] The *posterior* end of a bean is that end of the hilum at which the caruncle lies. It is the end opposite the micropyle.

been made between these two varieties. Something over 40 cross-pollinated beans have been secured. Of these, 15 have been grown at least as far as the F_1 generation. These 15 plants gave a total of 295 F_1 beans. Except for some minor fluctuations these F_1 beans were all alike, but differed markedly from either parent. In the notes these F_1 beans have been designated "Piebald" because of the very irregular spotted pattern. Fig 3 shows a typical piebald pattern. In addition to the spotting these beans differ from the Improved Yellow Eye in having a very irregular outline to the colored area. While the pattern is somewhat variable, there is never any difficulty in distinguishing this from the typical Improved Yellow Eye pattern.

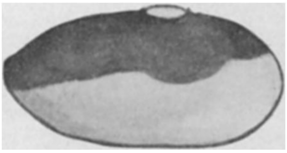

FIG. 1. Typical Improved Yellow Eye color pattern.

FIG. 2. Typical Old-Fashioned Yellow Eye color pattern.

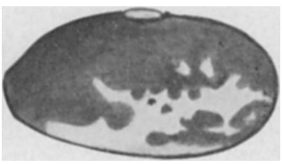

FIG. 3. Typical "piebald" color pattern occurring on the F_1 beans of a cross between the Improved and Old-Fashioned Yellow Eye types.

Up to the present time only a few of these hybrids have been carried to the F_2 generation. However, enough have been obtained to show that these piebald beans give both parent types and also more beans with the piebald pattern. It is very probable that only these three types occur in the F_2 and later generations.

While the data so far obtained from hand-pollinated hybrids are not sufficiently extensive to warrant further discussion, certain other data have been obtained which have a bearing on this subject.

In 1911 and 1912 the Experiment Station grew a number of plots planted with different strains and varieties of beans. Among these were a number of strains of Improved and Old Fashioned Yellow Eye. In some cases plots of these two varieties were located near each other. Seed from some of the 1911 plots were planted in 1912.

A considerable number of plants in these plots showed that the seed had been cross-pollinated by bumble-bees the year before. Among the plants in the Yellow Eye plots there were a number which bore typical piebald beans similar to that shown in Fig. 3. Some of these piebald plants were harvested separately and their progeny continued in a small way inside a screened cage. By the spring of 1915 it had been ascertained that a cross between an Improved and an Old-Fashioned Yellow Eye resulted in such a piebald pattern. Accordingly a considerable number of these piebald beans were grown in 1915. The following paper is based upon the data from these natural hybrids.

Table I gives the detailed data relative to the offspring

TABLE I

SHOWING THE SEGREGATION IN THE PROGENY OF PIEBALD BEANS

Pedigree No.	Year	Row	Piebald	I. Y. E.	O. F. Y. E.
1294–5	1912	30	11	3	4
	1913	87	—	3	2
		88	5	—	—
	1914	31	4	—	—
	1915	58	2	3	4
		269	12	7	3
		270	7	2	5
		271	8	1	3
		272	10	3	1
		273	6	—	2
		274	6	3	6
Total for 1294–5			*71*	*25*	*30*
1311	1913	104	3	—	—
	1914	32	2	1	2
	1915	292	13	7	4
		295	10	4	5
		296	6	1	3
		297	3	2	3
Total for 1311			*37*	*15*	*17*
153 X	1915	303	5	3	4
		304	8	1	3
Total for 153 X			*13*	*4*	*7*
1318	1915	307	5	2	5
		308	5	1	4
Total for 1318			*10*	*3*	*9*
1321	1915	310	8	3	3
		311	7	3	4
Total for 1321			*15*	*6*	*7*
Grand total			146	53	70

of 269 piebald beans. It will be understood that each row was planted from the offspring of a single plant. Not all of these beans can be considered as belonging to the F_2 generation. A portion of these certainly belong to the F_3 and later generations. This question will be considered further in a later paragraph.

From this table it will be seen that only three kinds of beans were obtained from these piebald seed. These were piebald, Improved Yellow Eye and Old-Fashioned Yellow Eye. This fact, in connection with the evidence obtained from controlled pollinations as noted above, makes it practically certain that these piebald beans are hybrids between these two varieties of Yellow Eye beans.

Further, with the exception of three small rows none of these piebald beans gave evidence of breeding true. In each of these three cases some of the piebald beans have split in later generations. Thus in pedigree No. 1294–5 the 1914 Row 31 is the offspring of one of the five piebald plants in the 1913 Row 88. It seemed possible that this line was breeding true. However, the 1915 Row 58 is the offspring of a plant from Row 31 of the year before, and Row 58 gave all three types, so that both of the preceding rows must have been heterozygous. If larger numbers had been grown from the same seed they would undoubtedly have thrown all three types.

The evidence thus indicates that the piebald pattern is the expression of the heterozygous condition of the factorial difference between these two types of Yellow Eye beans. A similar conclusion was reached by von Tschermak (1912). He obtained spotted beans very similar to our "piebald" from crosses between eyed and white or eyed and solid color beans. These piebald beans were always heterozygous, throwing on the one hand a large eye with regular outline corresponding with our Improved Yellow Eye and on the other hand a small-eyed bean. Judging from his figures (p. 208) von Tschermak's small-eyed bean had nothing corresponding to the peculiar pattern on our Old-Fashioned Yellow Eye. However, in

relative quantity of pigment these beans agree very well.

Von Tschermak assumed a unifactorial difference between the large and small-eyed beans, with the spotted pattern as the heterozygote. In the F_2 generation he obtained a 1:2:1 ratio.

Returning now to our own data as given in Table I it is clear that if the difference between the Improved and Old-Fashioned patterns is due to a single factor we should expect in the segregating generations 2 piebald:1 I. Y. E. :1 O. F. Y. E. The numbers obtained in Table I will hardly support this view. 146:53:70 can hardly be looked upon as a 2:1:1 ratio. It is true that the deviation is not so great, but that these observed numbers might be chance fluctuations from a 2:1:1 ratio. On the theory of probability the odds against the occurrence of such a deviation are about 5 to 1.

Of the more common Mendelian ratios the observed figures are much more closely fitted by 9:3:4. The observed and expected numbers in this case are

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	146	53	70
Expected No. on 9:3:4 ratio	151.3	50.4	67.3

It is clear that there is a very reasonable agreement.

Further evidence in support of the view that the segregation is not 2:1:1 is found by examining Table I in more detail. Thus the totals for each of the five pedigrees show an excess of Old-Fashioned Yellow Eyes over the Improved type. In three of these pedigrees the number of plants is relatively small. However, the cumulative evidence makes it almost certain that the deviations are not due to chance.

It was stated above that only a portion of these plants belonged to the F_2 generation. In a bifactorial character considerable difference might be introduced by the combination of data from different generations. From the records it is known that all the plants in pedigree Nos. 153 X, 1318 and 1321, together with two rows, 104 and 292,

from pedigree 1311, belong to the F_2 generation. Taking these plants alone, we have the data given in Table II.

TABLE II

Showing the Segregation in the F_2 Generation from Piebald Beans

Piebald	I. Y. E.	O. F. Y. E.
54	20	27

It is seen at once that there is again the same relative excess of O. F. Y. E. over I. Y. E. that is shown by the complete data in Table I. The expectation on the 2:1:1 ratio is 50.5:25.3:25.3, while on the 9:3:4 ratio the expectation is 56.8:18.9:25.3. It will be seen that the latter figures more nearly fit the observed numbers.

A 9:3:4 ratio presumes a bifactorial composition. However, a moment's consideration shows that such a ratio cannot have its usual significance in this case. If this were the usual bifactorial segregation, one out of every nine F_2 piebald beans ought to breed true in the third generation. Yet out of 15 rows from piebald beans which certainly belong to the F_3 or F_4 generation not a single one bred true.

Further, one half of the F_2 Old-Fashioned Yellow Eye segregates and two thirds of the F_2 Improved Yellow Eye segregates ought to show segregation in the third generation. In 1915, 43 Old-Fashioned Yellow Eye, F_3 plants were grown and every one bred true. At the same time 38 F_3 plants were grown from Improved Yellow Eye seed. Thirty-seven of these gave typical Improved Yellow Eye beans, but one plant gave piebald beans. The F_2 plant which furnished this latter seed was grown without any protection from insects in 1912 and it is very probable that the one I. Y. E. bean which gave piebald seed was due to insect pollination with Old-Fashioned Yellow Eye pollen. This is all the more probable because the ratio 1:37 is by no means what would be expected on the usual bifactorial hypothesis.

The evidence is fairly conclusive that the I. Y. E. and the O. F. Y. E. segregates breed true and that beans with

the piebald pattern are always heterozygous. These results could be very simply interpreted on a single-factor hypothesis, but the numerical results do not fit the 2 : 1 : 1 ratio demanded by that hypothesis.

While the data at hand are not as extensive as one might desire in order to build a complete theory, yet there is much to be said in favor of the following provisional hypothesis. Let I be a factor which in its homozygous condition II produces the Improved Yellow Eye pattern. Then Ii will be the zygotic constitution of the piebald plants and ii that of the Old-Fashioned Yellow Eye pattern. Assume further a lethal factor L independent in its segregation and of such a nature that LL in the presence of II produces a non-viable zygote. The complete F_2 segregation would then be as follows:

1	$I\ I\ L\ L$	Non-viable[3]
2	$I\ I\ L\ l$	I. Y. E.
1	$I\ I\ l\ l$	
2	$I\ i\ L\ L$	
4	$I\ i\ L\ l$	Piebald
2	$I\ i\ l\ l$	
1	$i\ i\ L\ L$	
2	$i\ i\ L\ l$	O. F. Y. E.
1	$i\ i\ l\ l$	

Such a segregation would result in the ratio 8 piebald: 3 I. Y. E. : 4 O. F. Y. E. Testing this ratio against the total observed numbers in Table I we get

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	146	53	70
Expected No. on 8 : 3 : 4 ratio	143.5	53.8	71.7

It is seen that there is a very close agreement between the observed and expected numbers; much closer, in fact, than in the case of the 9 : 3 : 4 ratio previously used.

[3] The same result would be obtained if ll in the presence II produced a non-viable zygote. This point could be determined by suitable crosses between the F_2 segregates.

In the case of known F_2 plants, as given in Table 2, the results are

	Piebald	I. Y. E.	O. F. Y. E.
Observed No.	54	20	27
Expected No. on 8 : 3 : 4 ratio	53.8	20.2	26.9

Here again there is a very remarkable agreement. In fact all of the data at hand fit into this theory very nicely. Final proof of its correctness or incorrectness can only come with more extended crossings between the segregates and with the parent stocks. Such experiments are now under way.

Relation of Eye Patterns to Type of Vine

Two years ago while going over some data from pure lines of Yellow Eye beans grown inside a screened enclosure the writer was struck by the fact that with few exceptions all of the O. F. Y. E. pure lines had the bush type of vine, while nearly all the I. Y. E. lines were classed as short runners. This point was further emphasized by the observation that in several cases the segregation from piebald beans showed that all of the O. F. Y. E. segregates were bush beans and all the I. Y. E. were runners. It was, therefore, of some interest to tabulate the data relative to type of vine in connection with the eye pattern.

The classification of plants with reference to type of vine has usually been made at the time of harvest. In some years the plants grown inside the screened cage have been classified as to vine type shortly before harvest. In either case the plants were mature or practically so. The plants grouped under the term "bush" are those which show determinate growth, terminal inflorescence, and lack the ability to twine about supports. The "runner" plants show axillary inflorescence and the twining habit (circumnutation). All of the runner beans considered in this paper are of the short runner or short pole type, rarely reaching a total height of more than 125 cen-

timeters. Usually they develop few branches. Under ordinary conditions such beans do not show indeterminate growth. However, from the investigations of Emerson (1916) it is probable that they would do so if growth were not stopped by unfavorable conditions or excessive seed production.

Data as to type of vine are available from 247 of the plants given in Table I. Table III shows the distribution of the type of vine for each of the three eye patterns. The data for each pedigree number are summarized separately.

TABLE III

DISTRIBUTION OF TYPE OF VINE FOR EACH OF THE THREE EYE PATTERNS

Pedigree No.	Piebald		I. Y. E.		O. F. Y. E.	
	Runner	Bush	Runner	Bush	Runner	Bush
1294–5	40	19	12	6	0	27
1311	18	19	4	11	0	17
153 X	3	10	2	2	0	7
1318	0	10	0	3	0	9
1321	9	6	6	0	0	7
Total	70	64	24	22	0	67

The most striking thing in connection with this table is the complete absence of runner vines among the Old-Fashioned Yellow Eye beans. Apparently the gene for bush type of vine is closely associated with the gene for the Old-Fashioned Yellow Eye pattern. That this association is not absolute under all conditions is indicated by the fact that I now have two strains of Old-Fashioned Yellow Beans of unknown origin which for several generations have bred true to a distinct runner type of vine. A number of crosses have been made using these runner types of Old-Fashioned Yellow Eye. It is hoped that these and other experiments which have been started will throw some light upon this question.

Attention may be called to the apparent 1:1 ratio of runner to bush in the case of the piebald and Improved Yellow Eye beans. Emerson (1904, 1916), von Tschermak (1904, 1912) and others have shown that in crosses between tall (runner) and dwarf beans the expected F_2

ratio is 3 tall to 1 dwarf. Obviously the present data are of little use in the study of this question because in the first place it consists of a mixture of F_2, F_3 and F_4 plants and in the second place the vine characters of the parents in the different crosses are unknown. It is quite possible that the parents in the case of pedigree No. 1318 were both of the bush type. The 22 plants in the F_2 generation in this strain are all of the bush type.

The only reason for presenting the data in Table III at this time is to call attention to the relation between the bush type of vine and the Old-Fashioned Yellow Eye pattern. There seems to be no question but that these two characters are closely associated.

LITERATURE CITED

Emerson, R. A. 1904. Heredity in Bean Hybrids. Ann. Rept. Nebr. Agr. Expt. Stat. 1904, pp. 33–68.

1916. A Genetic Study of Plant Height in *Phaseolus vulgaris*. Nebr. Agr. Expt. Stat. Research Bull. No. 7, pp. 1–72.

Pearl, R. and Surface, F. M. 1915. Studies on Bean Breeding. I. Standard Types of Yellow Eye Beans. Ann. Rept. Me. Agr. Expt. Stat. 1915, pp. 161–176.

von Tschermak, E. 1904. Weitere Kreuzungsstudien an Erbsen Levkojen und Bohnen. *Zeit. landw. Versuchsw. Osterreich*, pp. 1–102.

1912. Bastardierungsversuche an Levkojen, Erbsen und Bohnen mit Rücksicht auf die Faltorenlehre. *Zeit. induk. Abst. Vererb.*, Bd. 7, pp. 81–234.